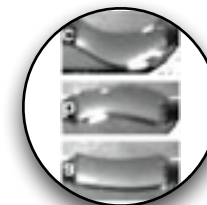
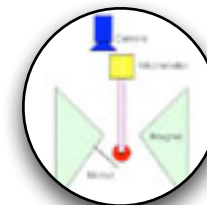
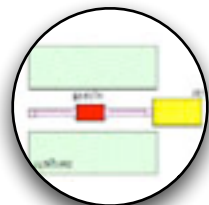


CHAPTER 5

BIOLOGICAL PHYSICS



Measurement Techniques for the Study of Dynamic Effects in Phospholipid Surfactants

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Problem: How to measure dynamic surface tension of pulmonary fluids

- Existing methods require large quantities of fluid and are affected by gravity
- Results difficult to interpret

Motivation

- During respiration alveoli grow and shrink periodically
- This requires *dynamic* variation of surface tension to balance $\Delta P = \frac{2S}{R}$
- Premature infants have not manufactured sufficient surfactant (e.g., phosphatidylcholine). Absence of these surfactants is thus life-threatening, particularly in neonates.

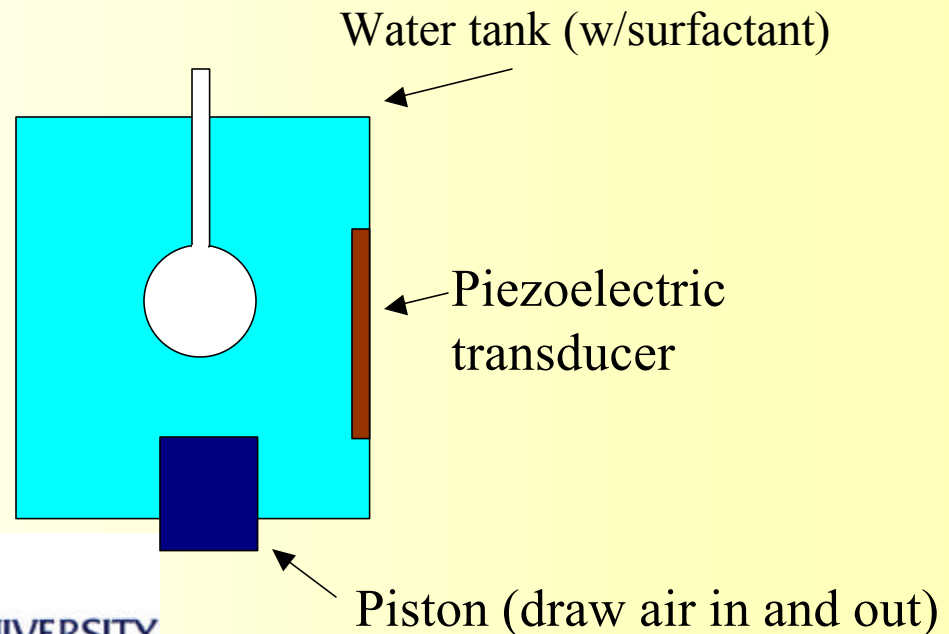


Extant techniques to measure dynamic behavior of surface tension

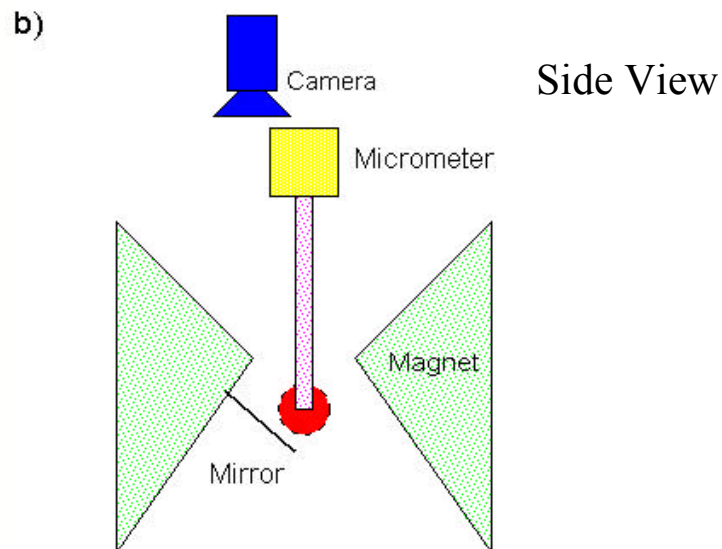
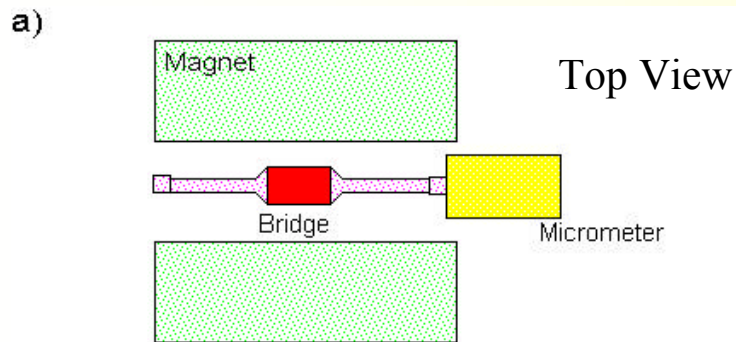
Existing methods require large quantities of fluid and are adversely affected by gravity

- Langmuir-Wilhelmy balance is very slow, involves flat surface, and is subject to gravity
- Pulsating Bubble technique (similar problems)

$$\Delta P = \frac{2s}{R}$$



Proposed solution: Use horizontal fluid bridges



Use *magnetic levitation* to simulate a low gravity (μg) environment and create quasi-cylindrical liquid columns in air.

Then, as a function of surfactant concentration:

either

I. Modulate magnetic force around zero Bond number and examine shifts in the resonance behavior

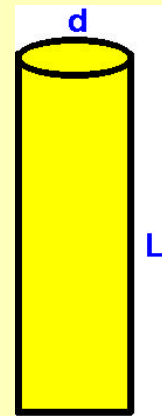
or

II. Rapidly reduce bridge length in zero gravity and examine the electrical resistance of the bridge. (Only force is surface tension)

We have used approach II

Aside: Liquid bridge primer

- Liquid bridges: Columns of liquid supported by two solid surfaces — These are generally opposing right circular cylinders.
- For a *cylindrical* bridge of length L and diameter d , **in zero g**, the maximum slenderness ratio $\Lambda [L/d] = \pi$ [Rayleigh]
- In the presence of gravity the cylindrical shape of bridge tends to deform



Liquid Bridge experiments are performed typically in:

- **Plateau (neutral buoyancy) tank** [problem: does not permit an air-liquid interface]
- **Drop tube** [problem: insufficient time for measurement].
- **Space-borne microgravity environment** [problem: does not facilitate easy variation with time of total body force. Otherwise, space is ideal for mg experiments].



These difficulties may be circumvented with our:

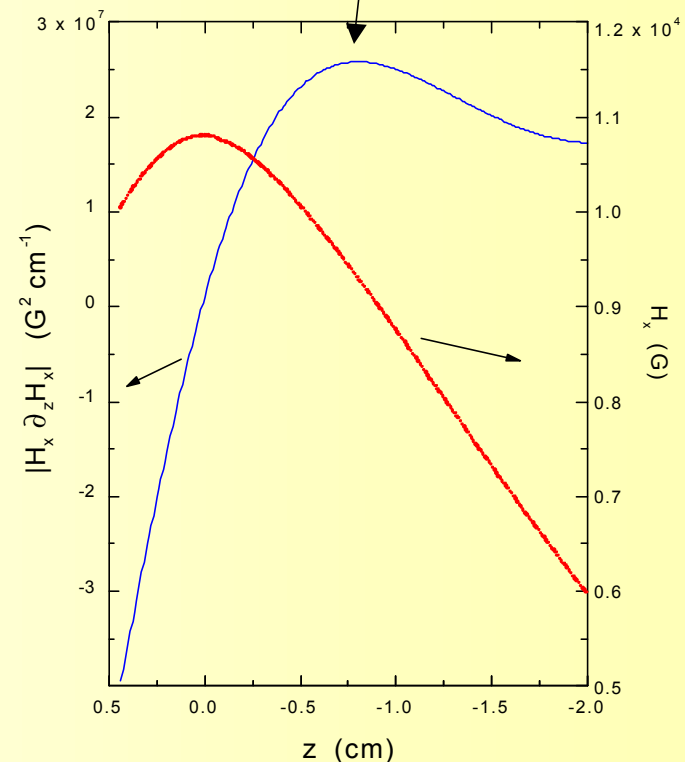
- **Magnetic levitation technique**

[This allows long duration measurements to be performed in air, and allows time variation of the total body force]

Principles

- Fluid has a volumetric magnetic susceptibility χ . On applying field H :
- Energy per unit volume is $U = -\frac{1}{2}\chi H^2 \rightarrow$
- **Force per unit volume is $F = -\tilde{N}U = \frac{1}{2}c\tilde{N}H^2 = cH\tilde{N}H$.**
- To compensate gravity, H_{comp} is the field whose gradient just compensates gravity.

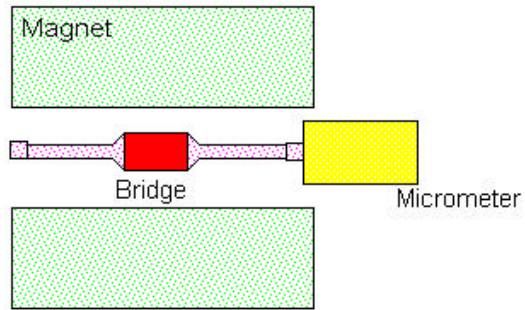
Location in magnet where the force is nearly spatially uniform and can be adjusted to compensate gravity



H and $H\nabla H$ profiles

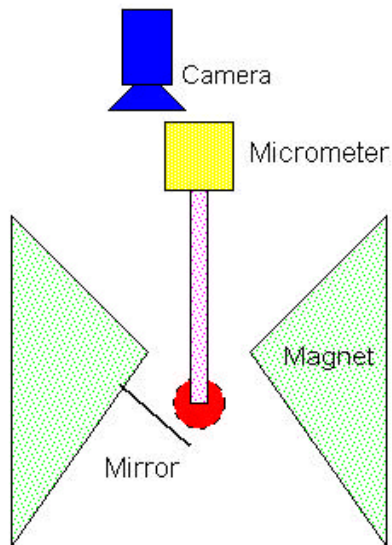
Experimental Apparatus

a)



Top view of horizontal bridge

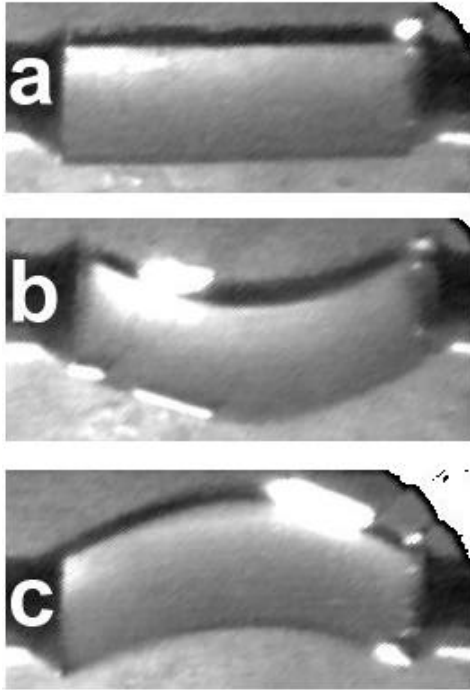
b)



End view of horizontal bridge

Materials

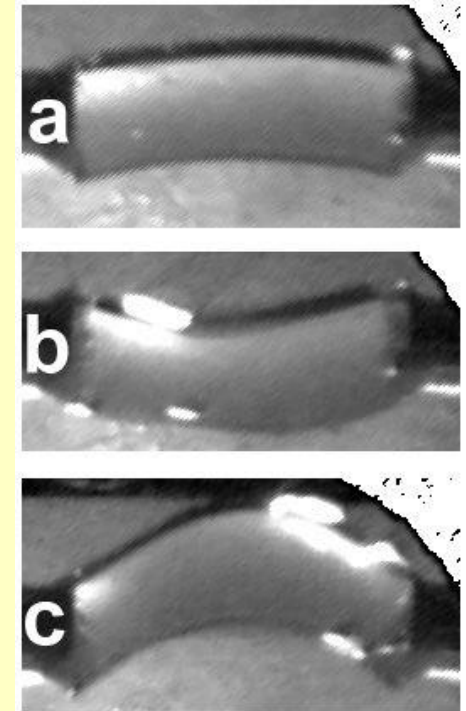
- Highly paramagnetic manganese chloride tetrahydrate
- Prepare mixture of 62.5 wt.% $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ in distilled water
- By weighing a known volume of the mixture, its density was determined to be $\rho = (1.45 \pm 0.01) \text{ gm cm}^{-3}$.
- Add small concentrations of model surfactant dodecyltrimethylammonium chloride (DTAC) (up to 1.5 wt.% of total mixture)



$$\Lambda = 2.58, B_{\text{eff}}^0 = 0$$

$$\omega = 25 \text{ s}^{-1}$$

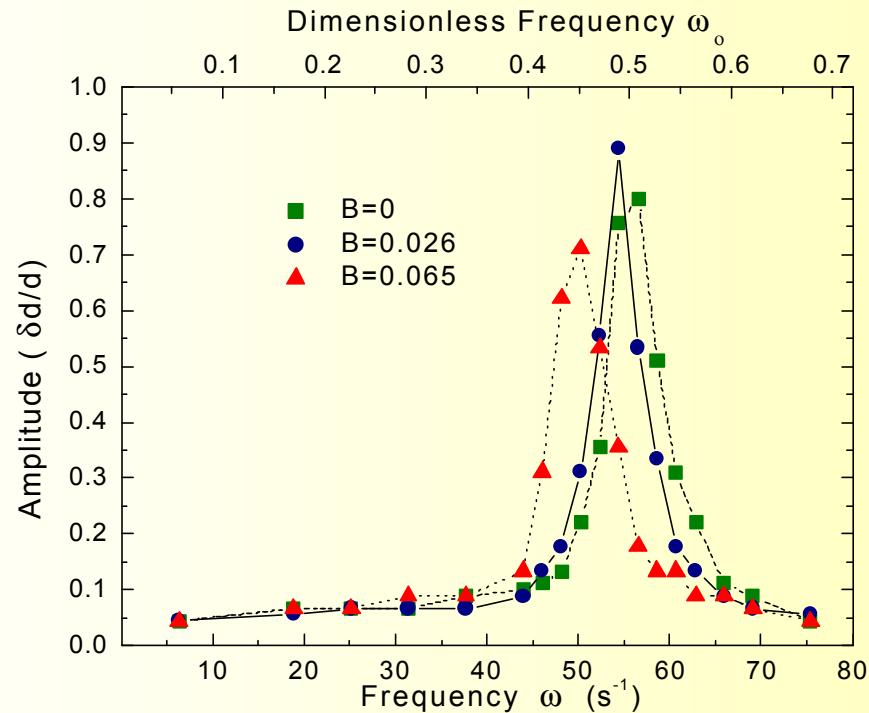
Views of horizontal bridge under *oscillating* magnetic field conditions.



$$\Lambda = 2.58, B_{\text{eff}}^0 = 0.065$$

$$\omega = 25 \text{ s}^{-1}$$

B is the Bond number — the ratio of total body force (gravity+magnetic) to the surface forces. We control B by varying the magnet current. Note that $B=0$ corresponds to zero total body force (an effectively gravity-free environment).



Resonance behavior of bridge as a function of Bond number.

Resonance behavior also may be used to examine dynamic surface tension. (This is Approach I). This is a subject for future investigations.

We have used Approach II: Transient Dynamics of Liquid Bridges by Studying *Shape Changes* in Microgravity

When gravity has been completely compensated by an upward magnetic force, the equilibrium shape of a liquid bridge in microgravity is determined by its surface tension only.

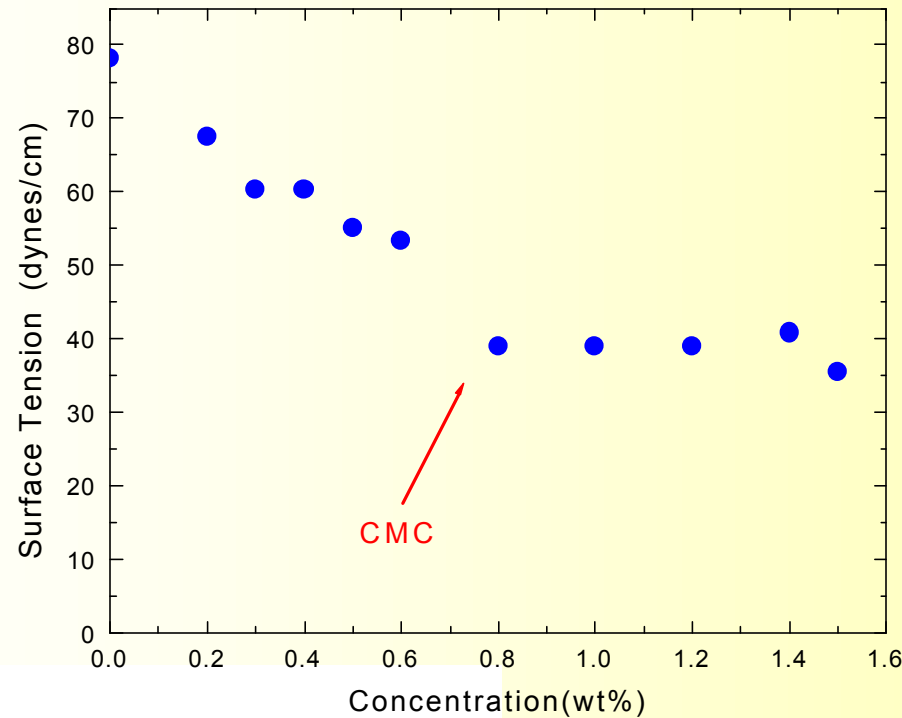
- Make sudden change in bridge length and study dynamic approach to equilibrium shape.
- Use *electrical resistance* measurements to monitor ongoing shape change.



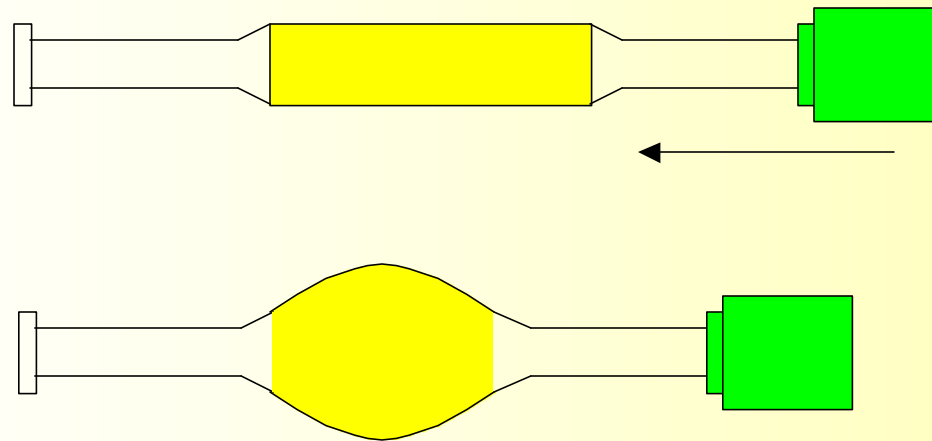
- Mixtures of paramagnetic liquid ($\text{MnCl}_2 \cdot 4\text{H}_2\text{O}/\text{Water}$)
- Add Dodecyl trimethyl ammonium chloride (cationic surfactant)

$$0 \leq X \leq 1.5 \text{ wt. \%}.$$

- Critical Micelle Concentration (CMC) is determined from surface tension measurements using capillary rise technique.



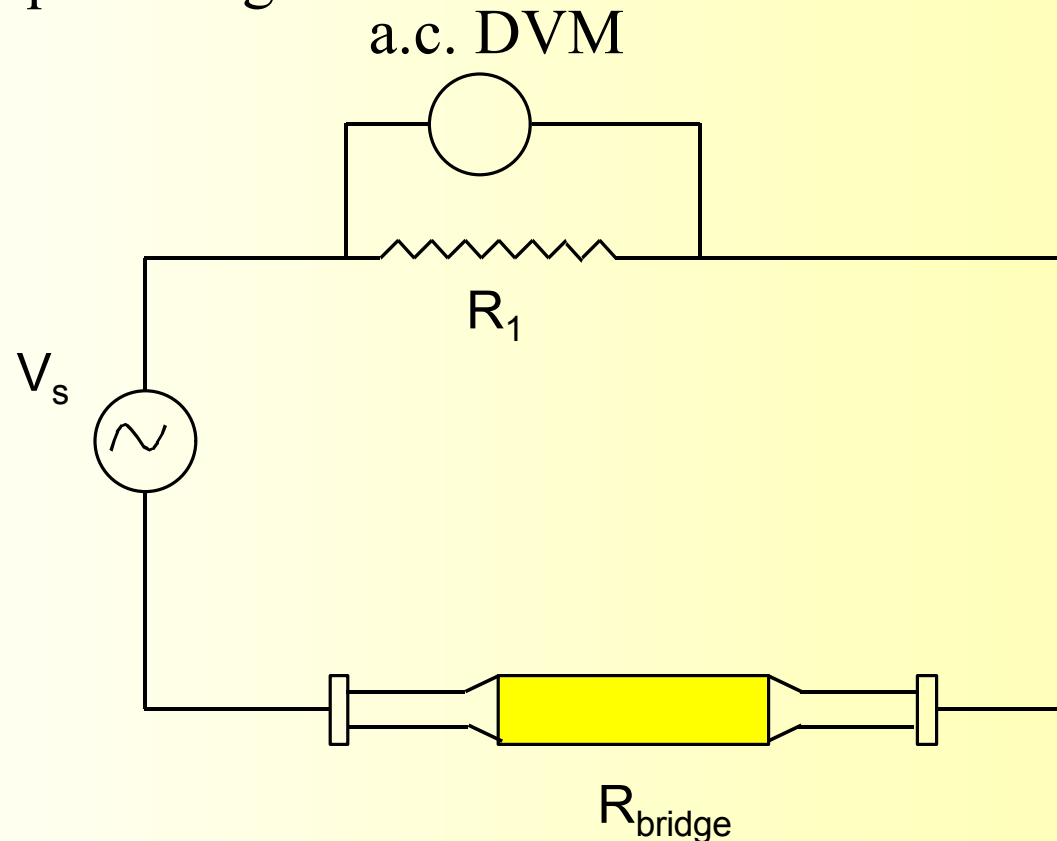
- For each concentration X of surfactant, bridges of $\Lambda = 2.5$ are created.
- A rapid change of length (1.3 mm in 500 ms) forces it to assume a new shape.



Conductance of the liquid bridge is measured vs. time @ 1000 Hz. Voltage across shunt resistor is proportional to the conductance of the liquid bridge.

$$Cond = \frac{A_{cross-section}}{rL}$$

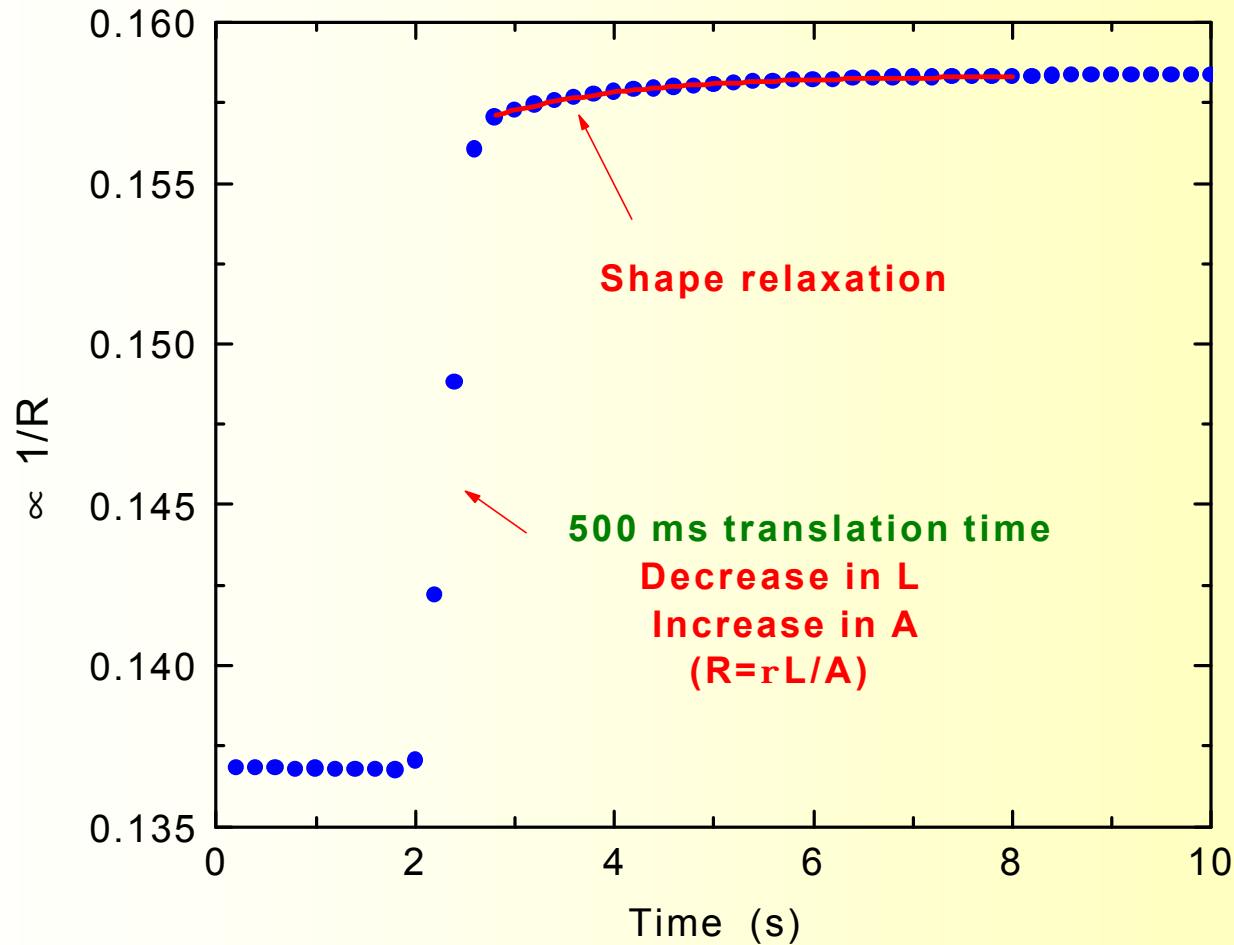
$$V = \frac{V_s * R_1}{R_1 + R_{bridge}}$$



Note: a.c. voltmeter cutoff frequency is ~ 6 Hz \rightarrow only *decay of envelope* due to bridge oscillations is observed.



Typical voltage signal measured:

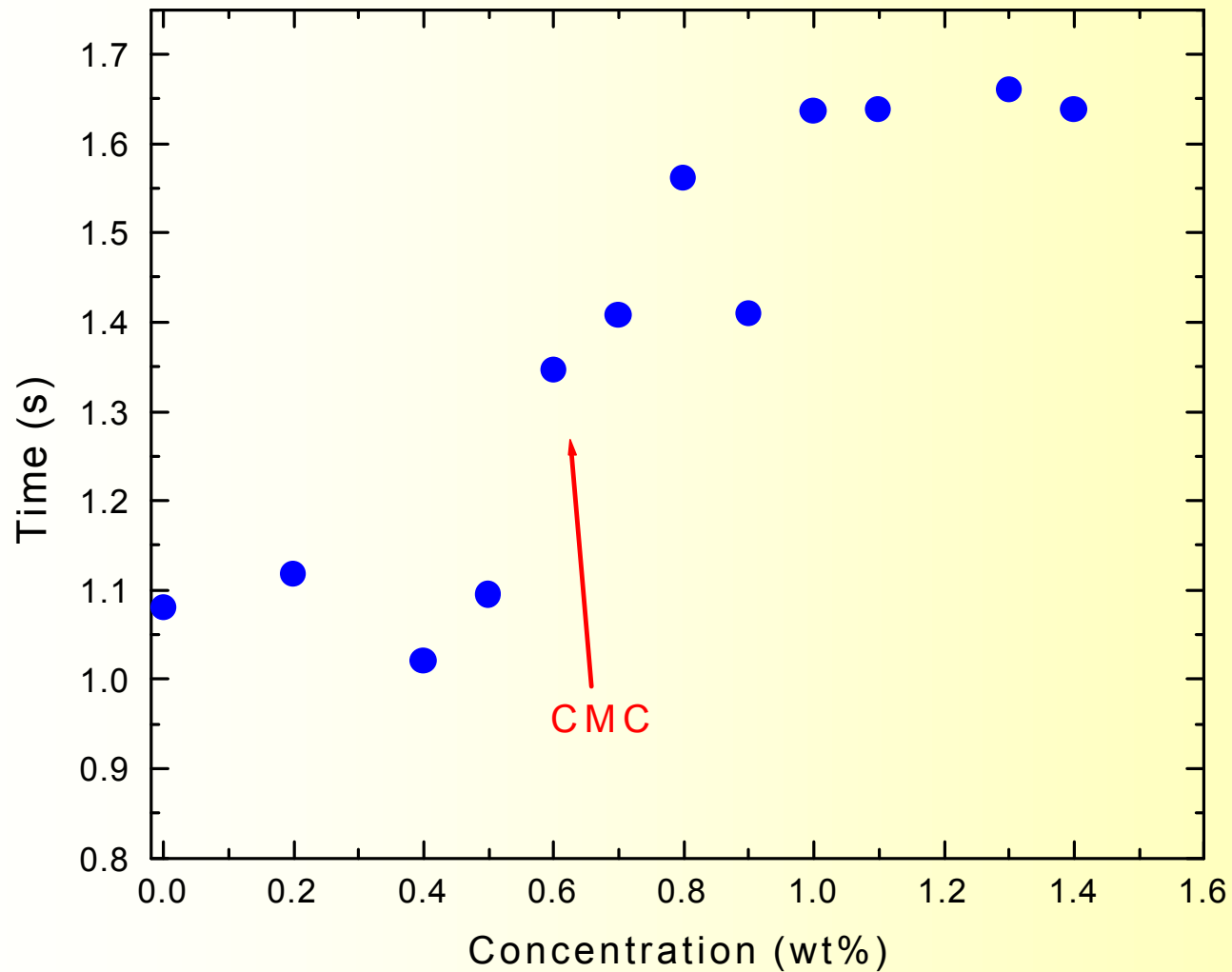


We are interested in the small amplitude shape relaxation to understand the surfactant dynamics



CASE WESTERN RESERVE UNIVERSITY

Measured relaxation time τ vs. X



In region $X < \text{CMC}$ ($\tau \sim 1.1 \text{ s}$)

- Surface area *decreases* on translation of rod. Increased surfactant density at surface *can* be accommodated by surface.
- Vibrationally-induced fast capillary waves ($> 8 \text{ Hz}$) keep resistance high (and therefore the measured voltage across the resistor is lower than its equilibrium value).
- As capillary waves decay, resistance decreases to final equilibrium value (associated with final equilibrium shape)
- *Observed decay time in this concentration region is related to capillary waves, not to changes in surfactant concentration at surface.*

In region $X > \text{CMC}$ ($\tau \sim 1.7 \text{ s}$)

- *Capillary waves are damped very rapidly for $X > \text{CMC}$, and do not contribute to measured signal during decay.*
- When rod translates, surface cannot rapidly accommodate higher surfactant density \rightarrow surface area is temporarily $>$ equilibrium area.
- Surface area relaxes from near equilibrium to equilibrium shape as surfactant is squeezed out from surface. Resistance relaxes with surface
- *In this concentration region, the decay time is related to the rate at which surfactant is squeezed out from surface. Thus, we have measured the effective expulsion rate of surfactant from the surface.*

Biological relevance: *We have developed a technique that can be used to examine the rate at which pulmonary surfactant may be expelled from the surface to the bulk.*

Next steps:

- *Examine behavior at higher concentrations*
- *Examine resonance peaks by varying the Bond number with time*
- *Examine biologically-relevant surfactants*
- *Examine behavior in the absence of manganese chloride tetrahydrate by using our 8.5T magnet for levitation of diamagnetic materials (e.g., water)*



Magnetic Field Effects in Magnetic Field Gradient Levitation of Biological Systems

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We are developing a Magnetic Field Gradient Levitation (MFGL) apparatus as a ground based system for simulating a low-gravity environment for biological and physical systems. This technique employs a strong, spatially varying magnetic field to exert a magnetic force on the diamagnetic materials in a system sufficient to oppose the earth's gravitational pull. Previous work with frog embryos has shown that MFGL is quite effective at canceling the body force of gravity on each of the constituents of levitated frog embryos. Here, we consider the fact that in addition to imposing a force field, MFGL also imposes a "torque" field that tends to align objects within biological specimens. Indeed, experiments have revealed that the magnetic alignment effect changes the early cell division geometry of frog embryos. This alteration depends on field direction and occurs for fields of magnitude comparable to those typically involved in MFGL. We present a model that explains the alterations in the cell division geometry in terms of a magnetic alignment affect. This model serves as an example of how to dissect and differentiate a "force" effect from a "torque" effect.

Improved flow cells for rapid mixing experiments using x-rays and fluorescence microscopy

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We have redesigned a previous version of a microfabricated flow cell to achieve two specific goals. The first is the optimization of signal to background for x-ray scattering experiments on both protein and RNA folding. To achieve this goal, we have nanofabricated ultra-thin silicon nitride windows for the sample cell. The second improvement allows sharper time resolution by creating a more homogeneous flow profile inside the mixing cell. Preliminary results of an RNA folding experiment, recently performed at the Cornell High Energy Synchrotron Source (CHESS) will be presented, showing that the new design significantly reduces the background signal.

The mixing cells have also recently been used to monitor rapid (microsecond scale) conformational changes in proteins using multi-photon and confocal microscopy. Preliminary results from experiments on fluorescently-labeled calmodulin will also be presented.